CLAIMS

1 A method of achieving enhanced expression of a target nucleotide sequence in a transgenic organism, which method comprises the steps of:
(i) providing an organism in which post-transcriptional gene silencing (PTGS) is suppressed,
(ii) associating said target nucleotide sequence with one or more heterologous Matrix Attachment Region (MARs), and
(iii) causing or permitting expression from the target nucleotide sequence in the organism.

2 A method of producing a transgenic organism in which a target nucleotide sequence is expressed at an enhanced level, which method comprises the steps of:
(i) providing an organism in which post-transcriptional gene silencing (PTGS) is suppressed,
(ii) associating said target nucleotide sequence with one or more heterologous Matrix Attachment Region (MARs), and optionally:
(iii) causing or permitting expression from the target nucleotide sequence in the organism.

3 A method as claimed in claim 1 or claim 2 wherein in (ii) two MARs are associated with the target nucleotide sequence in positions flanking it.

4 A method as claimed in any one of the preceding claims wherein the target nucleotide sequence is operably linked to a heterologous promoter or enhancer sequence.

5 A method as claimed in claim 4 wherein (ii) comprises the step of operably linking said target nucleotide sequence with a heterologous promoter or enhancer sequence.

6 A method as claimed in any one of the preceding claims wherein in (ii) the or each of the MARs is introduced to and associated with a target nucleotide sequence which is within a pre-existing gene present in the genome of the organism.

7 A method as claimed in claim 6 wherein the or each MAR is less than 500, 200, 150, 100, or 50 nucleotides upstream of a promoter or downstream of a terminator of the gene.

8 A method as claimed in claim 6 wherein (ii) comprises the steps of:
(iia) providing a target nucleic acid construct comprising (a) a promoter, and (b) one or more Matrix Attachment Regions (MARs) associated therewith,
(iib)introducing said target construct into a cell of the organism, such that the promoter becomes operably linked to a target nucleotide sequence which is within a pre-existing gene present in the genome of the organism.

9 A method as claimed in any one of the preceding claims wherein the target nucleotide sequence is endogenous to the organism.

10 A method as claimed in any of claims 1 to 5 wherein (ii) comprises the steps of:
(iia) providing a target nucleic acid construct comprising (a) an expression cassette including the target nucleotide sequence operably linked to a promoter, and (b) one or more Matrix Attachment Regions (MARs) associated therewith,
(iib) introducing said target construct into a cell of the organism,

11 A method as claimed in claim 10 wherein 1 MAR is associated with the expression cassette 5′ of the cassette.

12 A method as claimed in claim 10 wherein 2 MARs are associated with the expression cassette which flank the target nucleotide sequence.

13 A method as claimed in claim 11 or claim 12 wherein the or each MAR is less than 500, 200, 150, 100, or 50 nucleotides upstream of a promoter or downstream of a terminator of the expression cassette.

14 A method as claimed in any one of claims 8 or 10 to 13 wherein the target construct is a vector which comprises border sequences which permit the transfer and integration of the MARs into the organism genome.

15 A method as claimed in claim 14 wherein the target construct is a plant binary vector.

16 A method of transforming a plant cell involving introduction of a construct as claimed in claim 14 or claim 15 such as to cause recombination between the vector and the plant cell genome.

17 A method as claimed in claim 16 which comprises the step of regenerating a plant from the transformed plant cell.

18 A method as claimed in any one of the preceding claims wherein (i) comprises the step of suppressing PTGS in the organism.

19 A method as claimed in claim 18 wherein step (ii) precedes step (i).

20 A method as claimed in any one of the preceding claims wherein the organism in which PTGS is suppressed is one which is deficient in one or more genes required to support PTGS.

21 A method as claimed in any one of claims 1 to 19 wherein one or more genes required to support PTGS are subject to PTGS.

22 A method as claimed in claim 20 or claim 21 wherein the organism is a plant and the genes required to support PTGS are selected from: SGS2; SDE1; SGS3; SDE3; AGO1; WEX.

23 A method as claimed in any one of claims 1 to 19 wherein PTGS is suppressed by one or more viral suppressors of gene silencing.

24 A transgenic non-human organism obtained or obtainable by a method as claimed in any one of the preceding claims.

25 A transgenic organism as claimed in claim 24 in which a heterologous target nucleotide sequence is expressed at an enhanced level,

wherein the organism is deficient in one or more genes required to support PTGS,

which organism includes in its genome (a) an expression cassette including the target nucleotide sequence operably linked to a promoter, and (b) one or more heterologous Matrix Attachment Regions (MARs) associated therewith.

26 A method as claimed in any one of the claims 1 to 23 wherein expression is enhanced at least 5, 10, 15, 20, 25, or 30-fold.

27 A method for generating a target protein, which method comprises the steps of performing a method as claimed in any one of the claims 1 to 23 or claim 26 wherein the organism is a plant, and harvesting a

tissue in which the target protein has been expressed and isolating the target protein from the tissue.

28 A target nucleic acid construct for achieving enhanced levels of expression of said target nucleic acid comprising (a) an expression cassette including the target nucleotide sequence operably linked to a promoter, and (b) one or more Matrix Attachment Regions (MARs) associated therewith, when used in connection with a cell or organism undergoing suppression of PTGS.

29 The construct according to claim 28 wherein 2 MARs are associated with the expression cassette which flank the target nucleotide sequence.

30 The construct according to claim 28 or 29 wherein the target construct is a vector which comprises border sequences which permit the transfer and integration of the MARs into the organism genome.

31 A composition for use in a cell or organism which comprises a target nucleic acid construct for achieving enhanced levels of expression of said target nucleic acid comprising (a) an expression cassette including the target nucleotide sequence operably linked to a promoter, and (b) one or more Matrix Attachment Regions (MARs) associated therewith, when used in connection with a cell or organism undergoing suppression of PTGS.